On distribution of and hybridisation between the newts *Triturus vulgaris* and *T. montandoni* in western Ukraine

Spartak N. LITVINCHUK* **, Leo J. BORKIN* & Juri M. ROSANOV**

* Department of Herpetology, Zoological Institute, Russian Academy of Sciences, 199034 St. Petersburg, Russia

** Institute of Cytology, Russian Academy of Sciences, 194064 St. Petersburg, Russia

A case of natural hybridisation between the newts *Triturus vulgaris* and *T. montandoni* was recorded in the Maloye Opolie area, Lvov province, western Ukraine, where the latter species is represented by a population geographically isolated from the main range in the Carpathian mountains. A female hybrid, probably of the first generation, was identified by means of DNA flow cytometry. No hybrids were found in the Ukrainian Carpathians. Syntopic occurrence of *T. montandoni* and *T. vulgaris* in western Ukraine is discussed.

INTRODUCTION

The Montandon's newt, *Triturus montandoni* (Boulenger, 1880), is endemic to the Carpathian mountains. The species occurs in the Ukrainian Carpathians from 150 up to 2000 m above sea level (SZCZERBAK & SZCZERBAN, 1980). The range of *T. montandoni* is wholly surrounded by the range of *T. vulgaris*. However, both ranges are, in fact, parapatric. Nevertheless, the two species can coexist in the same water body. Such cases of syntopic occurrence have been reported in Romania (FUHN, 1963, ŞOVA, 1973, FUHN et al., 1976, our data), Slovakia (GULIČKA, 1954), Czech Republic (REHÁK, 1993), Poland (SZYMURA, 1974, JUSZCZYK, 1987) and in Ukraine (HORBULEWICZ, 1927, SZCZERBAK & SZCZERBAN, 1980, fig.1).

Triturus montandoni is genetically closely related to *T. vulgaris*, and both species have similar sexual behaviours (BELYAEV, 1979, 1981, PLEIO & RAFINSKI, 1985; RAFINSKI & ARNTZEN, 1987; ARNTZEN & SPARREBOOM, 1989). Therefore, it is not surprising that in the laboratory the two species can hybridise and that such crosses can provide fertile adult offspring (WOLTERSTORFF, 1925; GEYER, 1953 cited from FUHN, 1963, MACGREGOR et al., 1990; COGĂLNICEANU, 1994; our data).

Source : MNHN, Paris

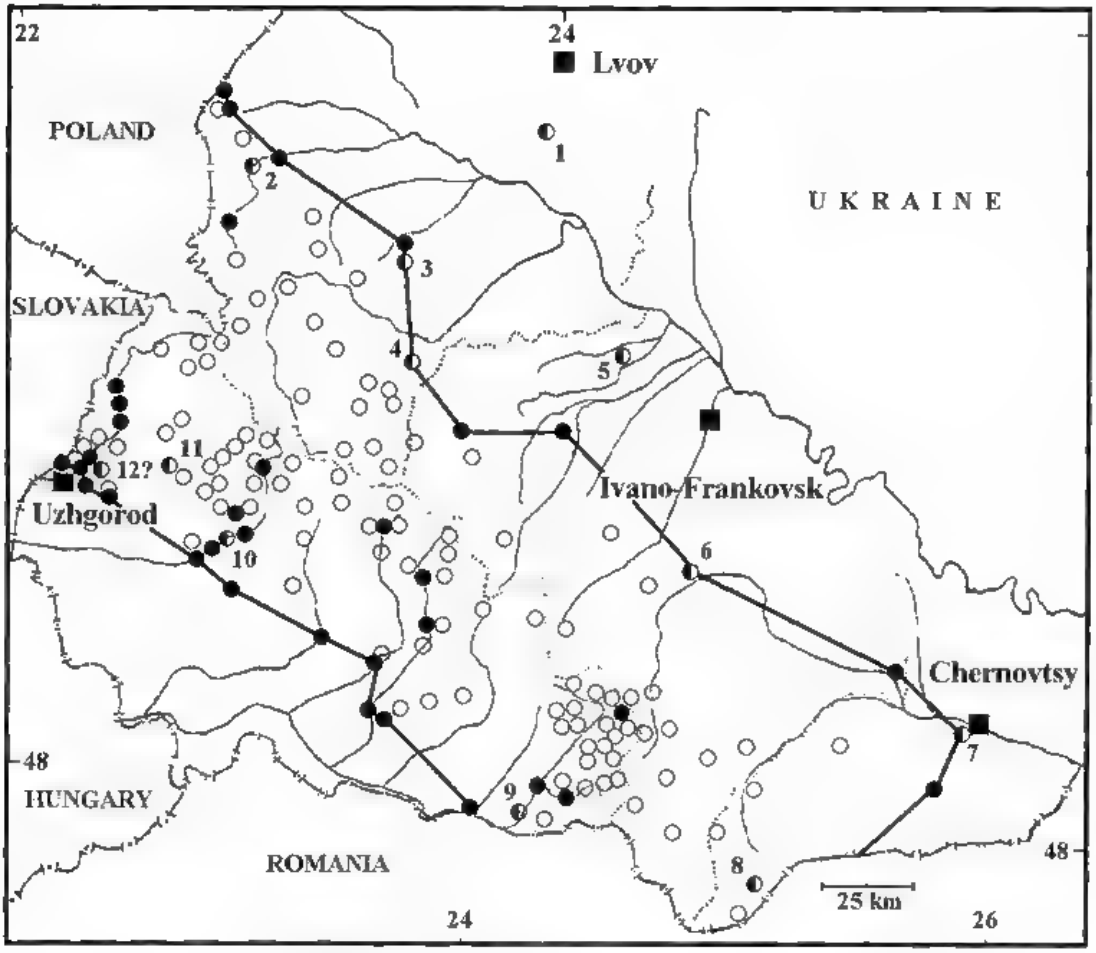

Fig. 1. Distribution of *Triturus montandoni* (open circles) in western Ukraine. The localities shared with *T. vulgaris* (semiopen circles) are: (1) Rakovets, 295-350 m; (2) Stary Sambor, 709 m; (3) Truskavets, 300-360 m; (4) Skole, 430 m; (5) Vorulov, 350 m; (6) Delyatin, 500 m; (7) Chernovtsy, 150-250 m; (8) Dolishny Shepot; (9) Delovoe, 365 m; (10) the Karpaty sanatorium, 165 m; (11) Poroshkovo; (12) Domanintsy. (13) Kriva. Sources: (1), (3) & (13), our data; (2) HOFMANN (1908); (3) POLUSHINA & KUSHNIRUK (1962); (4-8) & (11), SZCZERBAK & SZCZERBAN (1980); (9) O. A. Lugovoy & V. F. Pokinchereda (pers. comm.); (10) N. L. Orlov (pers. comm., ZMM 1404 & 2975, ZISP 5025, 5026 & 5561); (12) KUSHNIRUK (1963). Solid circles are sporadic localities of *T. vulgaris* in the Carpathians. The border of the main distribution of *T. vulgaris*, beyond the mountains, is marked by a black line.

In the breeding period, male hybrids obtained from laboratory crosses have a combination of various features: from *T. montandoni* - long tail filament (4 mm), well-developed dorsolateral ridges, black feet, gray throat without spots and bright orange belly; and from *T. vulgaris* - dorsal crest (height 1.5 mm) and small black dots on the belly. Female hybrids differ slightly from females of *T. montandoni*, although hybrids have small black spots on the belly.

In the field, several authors found newts with a pattern similar to that of the laboratory hybrids and recognized them as natural hybrids. For instance, such newts were reported from

Source : MNHN, Paris

western Ukraine (fig. 1: localities 2, 3 and 12), Romania (FUHN, 1963; FUHN et al., 1976) and Czech Republic (REHÁK, 1993). However, such records are uncommon, and in Slovakia, for instance, long term (16 years) observations on syntopic populations of *T. montandoni* and *T. vulgaris* provided no hybrids (GULIČKA, 1953).

An application of biochemical techniques could facilitate more reliable identification of such presumptive hybrids, based on external characters only. We know only two cases where interspecific hybridisation has been confirmed by allozyme analysis. In some syntopic localities in Poland, the incidence of hybrids, which were mainly recombinants, varied between four and 60 % (PLEIO & RAFINSKI, 1985, RAFINSKI, 1985). In a Czech locality where both species coexisted, five specimens with intermediate characters were collected (KOTLÍK et al., 1997). Based on three loci, four animals were identified as hybrids: one individual, perhaps, was a product of a backcross of the second generation and others were the offspring of more distant crosses. No hybrids of the first generation were found.

Triturus montandoni is protected in many countries. The species is listed in the Red Data Book of Ukraine although its local density can be quite high. Based on our data, *T. montandoni* is very common and obviously predominates over any other newt species in the Ukrainian Carpathians. COGĂLNICEANU (1997) suggested that, although not sufficiently documented, hybridisation with *T. vulgaris* may contribute to the reduction of its range and even pose a threat to its long term conservation.

The goal of our study was to investigate possible hybridisation between *T. montandoni* and *T. vulgaris* in western Ukraine.

MATERIALS AND METHODS

In 1989-1996 we searched for presumed hybrids between *T. montandoni* and *T. vulgaris* in all districts of Zakarpatskaya Province, as well as in Turka, Sambor, Khyrov, Drogobych, Nikolaev and Pustomyty districts of Lvov Province. In total we examined, mainly in the field, the external characters of above one thousand individuals of *T. montandoni* and *T. vulgaris* from western Ukraine including 567 individuals from the Maloye Opolie area (tab. 1). After careful examination, the animals were usually released to the same water body where they had been collected. Some animals, including 27 individuals from Maloye Opolie, were studied by DNA flow cytometry.

The amount of DNA per nucleus (genome size) was determined in relative units as a ratio of the fluorescence intensity of cells from an individual examined to that of reference cells. The details of the technique have been published by VINOGRADOV et al. (1990). Peripheral blood cells of *Pleurodeles waltl* were used as a standard.

Apart from our field study, we examined some museum collections. Museum abbreviations are: IZK, Institute of Zoology, Ukrainian Academy of Sciences, Kiev, Ukraine; ZISP, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; ZMM, Zoological Museum, Moscow State University, Moscow, Russia.

Source : MNHN, Paris

Table 1. – Occurrence of four newt species along the Rakovets gradients from the forest edge to the deep forest. Abbreviations: *T. m.*, *Triturus montandoni*, *T. v.*, *T. vulgaris*; *T. a.*, *T. alpestris*; *T. c.*, *T. cristatus*.

Waterbodies		Forest edge				600-700 m from the forest edge				2200-3000 m from forest edge			
Newt species		*T. m.*	*T. v.*	*T. a.*	*T. c.*	*T. m.*	*T. v.*	*T. a.*	*T. c.*	*T. m.*	*T. v.*	*T. a.*	*T. c.*
Date:	29.05.94	no data				123	3	19	3	no data			
	18.04.95	0	2	0	0	186	0	10	10	no data			
	11.05.96	2	0	0	0	144	0	23	2	102	0	35	0

Table 2. – Genome size variation in *Triturus vulgaris* and *T. montandoni*.

Province	*n*	Mean	Standard deviation	Range
T. vulgaris				
St. Petersburg	77	1.121	0.011	1.076-1.147
Zakarpatskaya	75	1.120	0.011	1.089-1.144
Lvov	7	1.111	0.011	1.096-1.127
T. vulgaris × *T. montandoni*				
Lvov	1	–	–	1.150
T. montandoni				
Lvov	19	1.182	0.009	1.167-1.199
Zakarpatskaya	6	1.176	0.011	1.158-1.189

Results and discussion

Having checked the zone of parapatry of *T. montandoni* and *T. vulgaris*, we found that some localities (fig. 1: 9 and 10) were inhabited by either of the two species only, although previous authors listed both species there. No morphological hybrids were identified.

On June 9, 1990, a sole locality with syntopic *T. montandoni* and *T. vulgaris* was recorded in the surroundings of Truskavets, Lvov province, near the town's water reservoir (fig. 1: locality 3). In a small round puddle (diameter 3 m, depth 15 cm) situated in an depression on the ground road nearby the forest, six individuals of *T. vulgaris* (3 males and 3 females) and a female of *T. montandoni* were found. Despite our attempts, no males of *T. montandoni* were observed there. Curiously, the only female of the latter species was transferred to St.

Source : MNHN, Paris

Petersburg and laid eggs successfully. Normal larvae hatched and metamorphosed. The juveniles had unspotted bellies and were similar to those of *T. montandoni*. Later these animals died for various technical reasons. Unfortunately, we failed to identify the presumptive hybrid origin of these young individuals.

In 1994-1996, our special attention was focused on an isolated population of *T. montandoni* from the surroundings of the village Rakovets, Pustomyty district, Lvov province, which is situated 30 km south of Lvov city (fig. 1: locality 1). This hilly area named Maloe Opolie is recognized by geographers as an extension of the Bobrko-Stolskoye Kholmogorie which belongs to the Podolskaya eminence. The area is partly covered by a small beech forest of the so-called Carpathian type which is separated from the beech forests of the Carpathian mountains by the valley of Dniester river over a distance above 50 air kilometers. This isolation seemed to be associated with changes in the Carpathian forest limits in the Atlantic epoch, i.e. 5-8 thousand years ago (MALINOVSKY, 1991).

This area is inhabited by all four newt species which are distributed in the Carpathian mountains, i.e., *T. vulgaris*, *T. cristatus*, *T. alpestris* and *T. montandoni*. The two latter species are obviously represented by populations geographically isolated from their main ranges in the Carpathians. BAYGER (1937, 1959) was the first to publish local records of *T. montandoni* and *T. alpestris*. He listed 38 localities covering the Maloye Opolie area as a whole (BAYGER, 1938 - cited from GULIČKA, 1954). Surprisingly, later in soviet time these Bayger's papers were forgotten, and the locality labels of his Opolie specimens kept in the Ukrainian museums of natural history in Kiev (e.g., a jar with the Rakovets sample of *T. montandoni*, IZK 80) and in Lvov (TARASHCHUK, 1959) were considered to be incorrect! However, relatively recently (in 1985), some localities mentioned by Bayger were repeatedly discovered by local researchers (S.V. Shaytan, pers. com. - cited from TATARINOV, 1989; POLUSHINA et al., 1989).

In 1994-1996, we monitored distribution of both species in Maloye Opolie along a gradient from the forest edge to deep forest which coincided with a gap arranged by forest cutting and used by forest trucks. Three sites were observed.

(1) The first one was situated on the military site nearby the forest edge (295 m above sea level). There were few water bodies (ditches, total length 10 m, width about 1 m, depth 0.5 m) with dense water vegetation and mosses. These water bodies were visited in 1995 and 1996: during our first visit only *T. vulgaris* was found, whereas in the second visit we observed both species and captured only *T. montandoni* (tab. 1). The densities of the two species were quite low.

(2) The second site was situated in the forest, 600-700 m from the edge, on a slope of a small hill (350 m above sea level). Here in 1994, in wheel-tracks filled by water, we found four newt species (tab. 1), with a predominance of *T. montandoni*. The latter species was characterized by high density: for instance, 123 individuals were collected in a wheel-track (length 25 m, width 0.6 m, depth 0.4 m). However, *T. vulgaris* was quite rare (two males and one female only). In 1994, we also captured a female which had some external features similar to those of *T. montandoni*, and black spots on the belly like in *T. vulgaris*. The study by flow DNA cytometry established that this female had a genome size (tab. 2) intermediate between those of both species. Interestingly, the lab hybrids of the first generation, obtained by us in the cross of a male *T. vulgaris* and a female *T. montandoni*, had a similar genome size. Therefore, we incline to identify this "morphological hybrid" female as a true first generation hybrid

Source : MNHN, Paris

between both species. The study of the second water body was continued in 1995 and 1996. However, we failed to find either *T. vulgaris* or new hybrids (tab. 1).

(3) The third site (350 m above sea level) was also situated in a wheel-track in deep forest, 2200-3000 m from the edge. We visited the water body in 1996 only and found only *T. montandoni* and *T. alpestris*. The former species was represented by some unusual individuals, e.g. by a female with a shortened body or by other animals (1.5 % of the Maloye Opolie sample) with partial or total lacks of black or orange pigmentation. However, such abnormalities in local animals seemed to have no association with any hybridisation between *T. montandoni* and *T. vulgaris*, because we found similar colour abnormalities in *T. alpestris* as well, with an incidence up to 2.3 %. In contrast, we observed no abnormal individuals of these species in the Carpathian mountains. We suggest that the appearance of such abnormalities may be explained by peculiarities of the isolated newt populations of Maloye Opolie.

Consequently, despite the "enclave" status of *T. montandoni* in the Maloye Opolie area, the local contact zone between this species and *T. vulgaris* seems to be very narrow. Only few individuals of the latter species penetrated into the forest water bodies predominantly inhabited by *T. montandoni*. RAFINSKI (1985) reported that in Poland hybrid individuals were more common in populations where *T. vulgaris* was more abundant rather than in populations with predominating *T. montandoni*. Our finding is in agreement with this observation because the sole Maloye Opolie hybrid was found in the site with very sparse presence of *T. vulgaris*.

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Source : MNHN, Paris

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